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Interaction of *Lactobacillus acidophilus* NCFM grown on different carbohydrates with human intestinal epithelial cells: Adhesion properties and roles of S-layer proteins

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Adhesion of probiotics to the gastrointestinal tract is considered to be an important criterion for colonization. *Lactobacillus acidophilus* NCFM (NCFM) is one of the well-defined probiotic strains isolated from humans and used in dietary supplements and yogurts. Although the adhesion process is complex and involves a variety of proteins, Surface layer (S-layer) proteins have been found to have roles in this adhesion. Combination of probiotics with emerging prebiotics has been shown to alter protein expression in a way that might change the functional properties of the probiotic.

The present study aimed at investigating effects of emerging prebiotics on S-layer protein expression and adhesion properties of NCFM. Adhesion differences by growth on the carbohydrates raffinose, cellobiose, and as control glucose were examined by using the human intestinal HT-29 cell line. 2-D gel electrophoresis, image analysis, and protein identification by mass spectrometry were performed to identify differentially expressed S-layer proteins under these growth conditions.

The results showed that NCFM grown on raffinose and cellobiose significantly increased adhesion to HT-29 cells 2.8 and 2.4 fold ($p<0.001$), respectively, compared to glucose. Protein identification revealed that expression of NCFM SlpX and SlpA proteins was significantly increased in raffinose and cellobiose cultures ($p<0.05$). In conclusion, increased level of S-layer proteins due to utilization of raffinose and cellobiose leads to increased adhesion of NCFM to human intestinal HT-29 cells.

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Characterization of immunomodulatory effects of probiotic bacteria in zebrafish model

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The zebrafish (*Danio rerio)*, already used for the study of the human immune system has an innate response similar to that of humans, representing a relevant model for studying the immunomodulatory properties of probiotic bacteria. The objective of this work is to develop a sensitive vertebrate *in vivo* screening system to assess the ability of probiotic bacteria to colonize the intestine and may also be used to rank the strength of their immunomodulatory effects.

A fish feeding protocol with probiotic bacteria and/or with inflammatory agent (TNBS) was established. The epifluorescence microscopy and transmission electron microscopy were used to visualize the location of GFP-expressing bacteria in the gut. The results show that *Lactobacillus casei* ATCC334 attaches to and colonizes the zebrafish intestine Moreover, inflammation response was assessed in the zebrafish by quantitative RT-PCR and the results show a decrease in the expression of genes encoding pro-inflammatory cytokines TNF-α and IL-1β following the probiotic treatment, suggesting that this bacterium is able to limit inflammation caused by TNBS. A decreased number of recruited macrophages is also demonstrated in the same conditions.

This study highlights the importance of this animal model to characterize the immunomodulatory effects of probiotic bacteria.